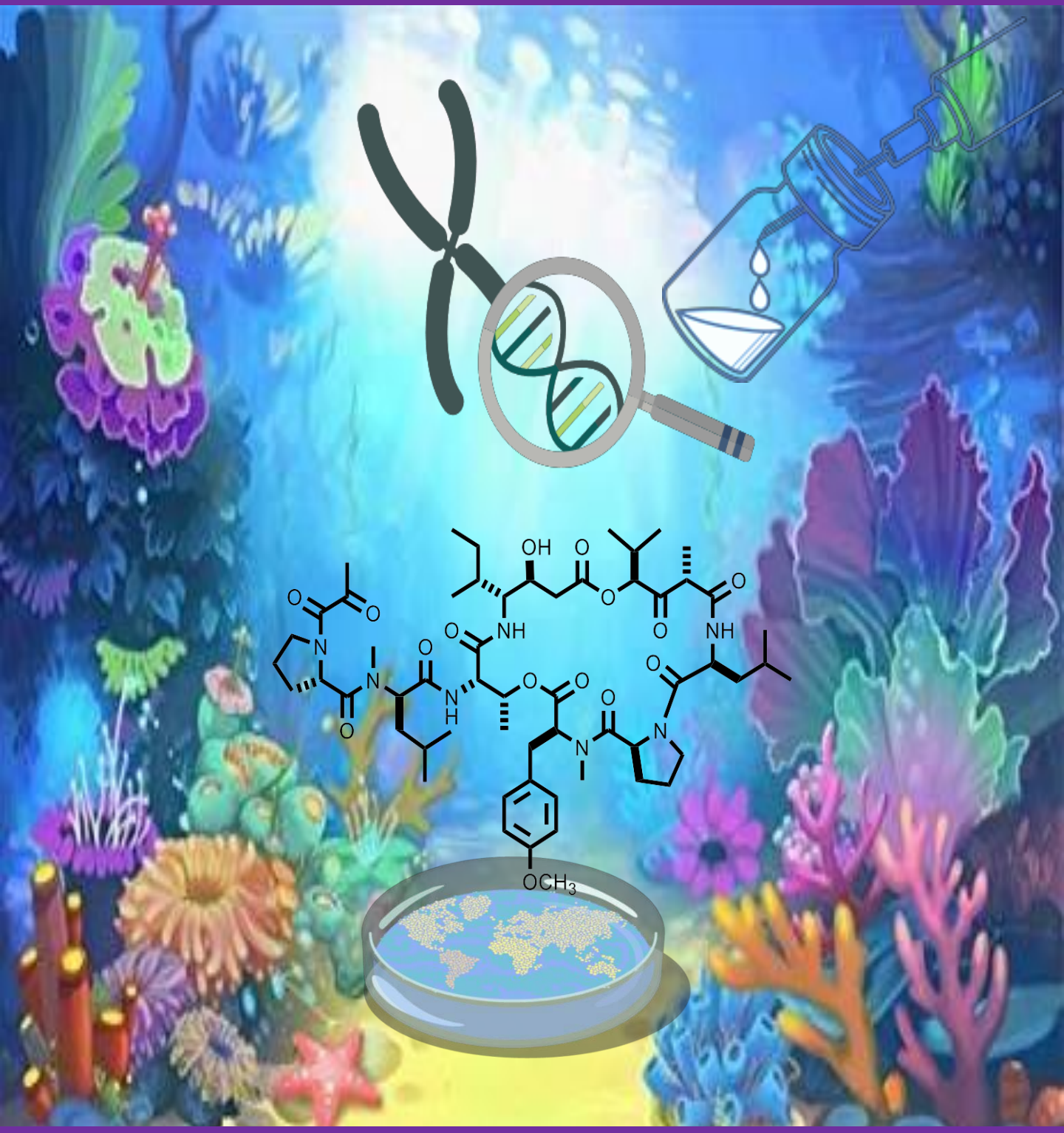
Marine Drugs Summary 2021/2022



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Marine Drugs - Summary

Need for Marine Natural Products Science

❑ By the end of the 20th century, disease resistance to antibiotics, to anti-viral, to anti-inflammatory and to anticancer drugs continued to grow at an alarming rate. Appearance of new diseases also has been noticed. As oceans represent more than 70% of the earth's surface and because marine environment characterized by new, exciting, and useful chemistry, Seas and Oceans were considered as a wealth source of bioactive natural products. Natural Products Chemists were increasingly turning to Marine Natural Products as a new source in the search for biologically and pharmacologically active compounds.

Marine Natural Products Science is a Modern Science

❑ Before early 1970th, there was no SCUBA Diving {SCUBA = Self-Contained Underwater Breathing Apparatus}, no submarines. In 1970th SCUBA Coupled with submarine vehicles, allowed relatively easy accessibility of both shallow and deep-water marine organisms for studies by natural products chemists. Marine natural products science is a modern science started in the late 1970th.

Divers can't go deeper than 100 feet in Ocean's water

❑ As if they are going deeper than that, they will suffer from Azotemia "Azote = Nitrogen emia = blood" a medical condition characterized by abnormally high levels of nitrogenous substances in the blood". Divers will suffer from narcotism, embolism and death.

Why Studying Marine Natural Products Branches

❑ It is important for marine drug discovery scientists to study Marine Natural Products branches. As after understanding the main ideas of each marine branch, they can inspire and discover new drugs from each branch.

Marine Natural Products Branches

A. Marine toxins: Toxic compounds isolated due to food poisoning e.g. Tetrodotoxin, a strong sodium channel blocker converted to a pain killer drug {Halneuron}. When taken by I.V, Halneuron is 3000 times more potent than morphine.

B. Marine Biopharmaceuticals: Bioactive compounds isolated for drug discovery e.g. Halichondrin B as anti-mitotic in breast cancer isolated from *Halichondria* sponge. Structure of Halichondrin B was modified {Structurally trimmed giving Eribulin - Halaven™} to decrease side effects and toxicity.

C. Marine Chemical Ecology: Interaction of marine creatures with each other e.g. Cone snail ω-conotoxin and paralysis of fish {Blocking Ca^{++} channels of the neurons in the spinal cord showing strong pain killing activity in chronic pain}.

D. Marine Biotechnology: Isolation of the gene clusters responsible for biosynthesis of certain bioactive compounds and transferring them to yeast, E. coli or to plant cells {transgenic cells} to produce large quantities of the active constituents in-vitro.

e.g. Transfer of gene cluster from diatoms producing omega-3 fatty acids with 7 un-saturations to *E. coli*.

Marine Creatures Taxonomy

❑ Marine Natural Products scientists must have at least a simple background about classification {Taxonomy} of marine creatures. As during marine samples collection each class of marine organisms contains certain chemical groups of active constituents with specific pharmacological activities e.g. Blue green algae contain polypeptides showing promising cytotoxic activity against cancer cells. Scientists will avoid samples known to contain inactive compounds.

Marine Sample Collection

❑ Collection of Marine Samples

- Samples of marine organisms near shores are collected by hand or by snorkeling.
- Samples of marine organisms up to 100 feet can be done by SCUBA diving.
- Samples of marine organisms more than 100 feet cannot be achieved by SCUBA , submarines may be used.

Why Extraction with Organic Solvents

❑ Marine creatures are keeping their pharmacologically active 2ry metabolites inside their bodies to defend themselves from enemies. They are not secreting their bioactive constituents continuously outside to water. These 2ry metabolic compounds are lipophilic {hydrophobic} compounds. In extraction step, sample homogenized then extracted with the suitable organic solvent e.g. ethanol, methanol, CH_2Cl_2, acetone and acetonitrile to dissolve the pharmacologically active organic compounds. If we use water for extraction, it will not dissolve these bioactive compounds.

Brine shrimp toxicity, ichtyotoxicity and fish toxicity

❑ Brine shrimp toxicity or ichtyotoxicity {Snail killing activity} or even fish toxicity are important steps in isolation of bioactive marine Drugs. If the extract or the fraction is toxic to {Brine shrimp - snails - Gold fish}, extract must contain strong pharmacologically active compound{s} that is/are toxic to Brine shrimps in large dose. But when used in diluted concentration, it can treat certain disorders e.g. Sodium channel blockers in large dose can cause respiratory paralysis and death to Brine shrimp, but in small doses can be used as local anesthetic or pain killer in chronic inflammation {e.g. Tetrodotoxin}.

Bioassay-guided fractionation

❑ Bioassay-guided fractionation {e.g. Cytotoxicity – COX I inhibitory activity} as a useful technique in science of marine drugs involves testing the crude extract or preliminary fractions from chromatography in an assay or multiple assays {Cytotoxic and Anti-inflammatory activities}, determining what fractions or crude extracts show activity in the

specific assays. Further fractionation of the active fractions or extracts is carried out and repeated where the new fractions are tested and the active fractions are further fractionated. This continues until the fraction only contains one pure compound {Bioactive compound}.

Structure Confirmation of Bioactive Marine Natural Products

❑ Structural elucidation of Marine bioactive compounds can be performed using:

- **Elemental analysis:** Gives percentage elements in the compound e.g. % C, % O, % N, % H. Also gives us the molecular formula of the compound.
- **Ultraviolet-visible spectroscopy** (UV-vis): gives an idea about the presence or absence of the conjugated unsaturated systems {Conjugated double bonds}.
- **Infrared (IR) spectroscopy**: gives an idea about the nucleous of the compound {fingerprint region}. Also gives an idea about functional groups e.g. OH, CH aromatic, C=O groups {functional group region}
- **MS spectroscopy:** Gives the molecular weight of the compound
- **NMR spectroscopy:**

  ^{1}HNMR gives us an idea about number and nature of the protons in the compound e.g. Carboxylic, aldehyde, aromatic and aliphatic.

  ^{13}CNMR gives us an idea about number and nature of the carbons in the compound e.g. carbonyl, Aromatic, aliphatic
- **X-ray crystallography:** Can identify the compound and its stereochemistry if the compound is available in crystalline form.

Bioactive Compounds Fail to be medicine in Clinical Trials

❑ To be approved as a medicine, a compound can take about 30 – 40 year from its discovery to be in the market. A medicine can cost up to 1 billion dollars. Many marine natural compounds failed in clinical trials phases as they may have unresolved problems in instability, insufficient supplying source, high toxicity and difficulty in total synthesis or semi-synthesis.

Marine Toxins

Tetrodotoxin (TTX)

❑ Tetrodotoxin (TTX) isolated from Puffer fish, octopuses and sea stars. The true producer of tetrodotoxin is a gram -ve organism *Pseudoaltermonas*, a micro-organism living on all of these creatures. Tetrodotoxin acts in large dose as a very strong Sodium Channel Blocker compound, leading to respiratory paralysis and death. Now it is manufactured by Wex Pharmaceuticals Canada and approved by Health Canada as a strong pain killer drug in cancer {Halneuron™} in very low concentration. It is 3000 times more active pain killer than morphine.

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Brevetoxin B and Ciguatoxin

❑ Brevetoxin B and Ciguatoxin (Polyether} exaggerate sodium influx {Voltage-gated sodium channel}. In large dose It triggers mechanism of bronchospasm, bronchial asthma, heart problems, abdominal pain, vomiting, diarrhea and cardiovascular disorders. Brevetoxin B and Ciguatoxin can be used in very dilute concentration as lead drugs in Syndrome of Improper Anti-Diuretic Hormone Secretion {SIADH}. They also have positive ionotropic activity {Heart rate}.

Maitotoxin

❑ Maitotoxin (Polyether} exaggerates calcium influx {Voltage-gated calcium channel} causing Vasoconstriction and cardiac arrest in large dose chronotropic effect}. In small dose it can be used as a lead drug to treatment hypotension

Palytoxin

❑ Palytoxin {Polyether nitrogenous compound} isolated from a number of marine organisms including the Coelenterate *Palythoa toxicus*. Palytoxin can be used as an alternative to digoxin {Digitalis} as it acts by inhibition of Na^+/K^+ ATPase {increases sodium influx through sodium pump positive ionotropic activity } also increasing Ca^{++} influx inside cells leading to muscle contraction {Cardiotonic activity chronotropic effect}.

Jellyfish toxins

❑ Polypeptides {main one is a 47 amino acids polypeptide} act by increasing K^+ influx in nerves and cells leading to severe irritation and edema in large dose {Treated by using Zinc acetate solution}. In low concentration Potassium is very important in regulating heart beats in arrhythmia, so Jellyfish toxins may be used in the future as lead drugs {pharmacophore} for treatment of cardiac arrhythmia by drug targeting.

Marine Pharmaceuticals

Chemical Diversity

❑ The nature of compounds characterized from marine organisms are diverse and represent many different structural classes, including linear Peptides {Dolastatin 10}, Cyclic Peptides {ω-Conotoxin}, Cyclic depsipeptides {Aplidin}, Nucleosides {Ara C - Ara A}, Polyether or Polyketides {Halichondrin B}, Alkaloids {Trabectedin}, PUFAs {Omega 3 fatty acids} and terpenoids {Pseudoterosine A}.

Peptides: Drugs of future

❑ Peptides are considered as the drugs of future as they have highly specific mode of actions, needed in low doses, have reduced risk of toxicity, used for bacterial infections, have diverse biological activities e.g. anti-inflammatory activity, anti HIV activity, antibiotic activity and anti-cancer activity.

Ribosomal polypeptides {RPPs} and Non Ribosomal polypeptides {NRPPs}

❑ Ribosomal polypeptides: Polypeptides biosynthesized by the routine transcription route {DNA contains gene cluster responsible for biosynthesis of peptides – DNA gives mRNA by translation - mRNA by translation gives polypeptides}.

❑ Non-ribosomal polypeptides {NRPP}: No gene cluster responsible for the biosynthesis of Polypeptides was found. It is said that: "NRPP are biosynthesized by a non-routine route which is constructed by Non ribosomal polypeptide synthases in the cytoplasm.

Precautions During Extraction, Purifications, Isolation and Use of Polypeptides

❑ Peptides need special precautions during extraction, purifications and isolation from marine creatures as they will be damaged {de-naturated} by high PH, low PH and high temperature {higher than 30 ^{0}C}. Extraction of Polypeptides from marine creatures must be carried out as soon as possible after collection of marine samples or from alcohol preserved organisms to inhibit enzyme activity and to prevent hydrolysis of polypeptides.

❑ Medical Polypeptides {Ziconotide} must be preserved in a lyophilized state at low temperature in a refrigerator and constituted in their solution for injection directly before use to avoid hydrolysis.

❑ Peptides with therapeutic value can't be administered orally nor by I.M injection as Plasma membrane restricts their movement across intestinal epithelial cells and also they are degraded by protease enzyme.

Depsipeptides

❑ Depsipeptides: Chain of amino acids {linked by amide groups} alternating with carboxylic acids {linked by ester group}.

❑ Detection of the depsipeptides in normal peptides:

- Using pepsin enzyme: Depsipeptides will not be completely digested {ester groups}
- IR: Carbonyl group in amide groups can be identified near the range of 1650 cm^{-1}. Carbonyl group in ester group can be identified near the range of 1730 cm^{-1}
- ^{13}CNMR: Can't differentiate between Carbonyl group in esters of depsipeptides and amide groups in all polypeptides {range from 165 – 175 ppm for both}

Isolation of Marine Polypeptides

❑ After extraction with organic solvents e.g. CH_2Cl_2/CH_3OH and upon using VLC {Vacuum Liquid Chromatography} for fractionations of marine organisms extracts, Polypeptides will be eluted mainly in the 80 % Ethyl acetate/hexane fraction and in the 100% ethyl acetate fraction {Mildly polar}.

❑ During isolation and purification of peptides with HPLC, PDA {Photodiode array detector = DAD} is more preferable than UV detector during separation of due to:

1- Wide range of λ_{max} {200 - 800nm}.

- DAD {PDA} detect nearly all the compounds in the range between 200-800 nm
- UV detector {Single wavelength} can detect peaks at one wavelength only e.g. 280nm
  UV detector with Variable wavelength detect peaks at multiple wavelengths 3 - 4 wavelengths e.g. 250, 280, 320nm

2- DAD {PDA} gives us an idea about peak purity of the compounds containing conjugated systems while UV detector can't.

Culturing of Marine Microorganisms

❑ The true source of most of the isolated Marine Natural Products compounds is the symbiotic microorganisms rather than the host. So it is important for Marine Natural Products scientist to know how to culture marine micro-organisms to isolate the bioactive compound or even the gene cluster from the true producer. Upon carrying out microbial culturing of the sea water micro-organisms, only 0.1% of the marine microorganisms will grow {due to differences in the conditions between sea water and nutrient media}. HTPS {**High Throuput Screening Technique**} can be used for growing microorganisms in short time and high numbers.

Identification of Marine Polypeptides

❑ They give blue to purple colour with Nin-hydrin

❑ **^{1}HNMR**: -**NH** of the amide group appears at 6.40 – 6.95 ppm one proton in integration
-N-CH_3 of the amide group appears at 2.85 – 3.00 ppm three protons

❑ For determination of molecular weight of the bioactive peptides, ESIMS {Electro Spray Ionization Mass Spectroscopy} and FABMS {Fast Atom Bombardment Mass Spectroscopy} are more preferable MS techniques in determination of the molecular weight and the sequence of amino acids as they are soft techniques giving gradual fragmentation of the peptides and cyclic depsipeptides. So we can know the molecular weight and predict the amino acid constituents and their sequence in the polypeptide easily.

❑ EIMS {Electro Impact Mass Spectroscopy} is a tough technique giving sudden and complete fragmentation of the cyclic peptides {e.g. complete damage of amino acids}. So we can't predict the amino acids and their sequence.

Detection of aromatic amino acids in psipeptides

- **UV spectroscopy:** Aromatic amino acids absorb electromagnetic waves in UV range as they have conjugated systems λ_{max} more than 280 nm.
- **IR** spectroscopy: C-H aromatic at 3000 - 3200 cm^{-1}
- **^{1}HNMR**: Aromatic protons chemical shift range 6.8 – 8.2 ppm
- **^{13}CNMR**: Aromatic carbons at 110 – 150 ppm

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D-amino acids and L-amino acids in polypeptides

❑ Contribution of the D-amino acids in polypeptides and cyclic depsipeptides make polypeptides toxic to man {D-amino acids will not be metabolized in human body}.

❑ Determination of the stereochemistry of amino acids in cyclic polypeptides can be carried out by hydrolysis of about 20ugm of the cyclic polypeptides with 6 N HCl in a vial then reaction with {**Marfey's reagent =** 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide}. Hydrolysis Product analyzed using chiral stationary phase HPLC using both standard L-amino acids and D-amino acids reacted with Marfey's reagent as references. Then compare retention data of sample amino acids and standard amino acids.

Marine Pharmaceuticals

❑ Compounds characterized from Marine organisms are diverse and represent many different structural classes, including Nucleosides, Polypeptides, cyclic depsipeptides, polyethers, terpenoids, alkaloids and PUFAs.

❑ These metabolites possess a wide spectrum of bioactivities, including anticancer activity, anti-inflammatory activity, antiviral activity and cardio-protective activity.

❑ Isolated marine biopharmaceutical compounds can be classified into the following major groups:

1Polypeptides {Cyanobacteria, tunicates, nudibranchs and snails} - Treat Cancer

2 Nucleosides {Sponges} – Treat Cancer and viruses

3 Alkaloids {Tunicates} – Treat Cancer

4 Polyketides {Sponges} - Cancer

5 - PUFAs = Polyunsaturated Fatty Acids {Fish} - Cardioprotective

6- Polyhalogenated monoterpens {Red algae} - Cytotoxic

7- Lipopeptides Amino acid + fatty acid {Cyanobacteria}

8- Sterols {Soft Corals}

9- Terpenoids {Soft Corals} - Inflammation

Approved Marine Drugs Cytarabine - Cytosar-U® - Ara C

Name: Cytarabine - Cytosar-U® - Ara C

Nature: Nucleoside {Arabinosyl cytosine}

Source: Caribbean marine sponge *Cryptotethya crypta*

Mechanism: Anti-metabolite inhibits DNA polymerase

Medical uses: Acute myelocytic leukemia

Vidarabine- Ara A

Name: Vidarabine

Nature: Nucleoside {Arabinosyl adenine}

Source: *Streptomyces antibioticus* {Used as antiviral}

Mechanism: Anti-metabolite inhibits DNA polymerase

Medical uses: *Herpes simplex* virus and *Varicella zoster* {Chickenpox virus}.

Brentuximab

Name: Brentuximab

Nature: Polypeptide monoclonal antibody conjugate

Source: Synthetic conjugate inspired from dolastatin 10

Mechanism: Microtubules stabilizer {anti-mitotic}

Medical uses: Lymphoma.

Aplidin – Plitidepsin

Name: Aplidin – Plitidepsin

Nature: Cyclic depsipeptide

Source: Tunicate *Aplidium albicans* {Now it is totally synthetic}

Mechanism: Acts as Protein kinase C Inhibitor = induction of apoptosis.

Medical uses: Used in Pancreatic cancer

Ziconotide {Prialt}

Name: Ziconotide {Prialt}

Nature: Cyclic polypeptide 25 amino acids inspired from ω-conotoxin

Source: Cone snail {*Conus magus*}.

Mechanism: Acts as a Ca^{2+} Channel blocker in the neurons of the spinal cord.

Medical uses: Strong analgesic in cancer and fractures. must be injected Intrathecal {Directly in the spinal cord} to prevent any loss of the compound in other body organs and to prevent its hydrolysis.

Eribulin – Halaven

Name: Eribulin – Halaven

Nature: Macrocyclic polyether {Polyketide}

Source: Synthetic compound inspired from Halichondrin B from *Halichondra* sponge.

Mechanism: Microtubule destabilization {Stop mitosis}.

Medical uses: Breast cancer.

Structural trimming

It is the removal of certain part of the bioactive compound during chemical synthesis to decrease side effects, increase selectivity, decrease toxicity or increase bioavailability e.g. Structural trimming of Halichondrin B was carried out to obtain Eribulin.

Belantamab Blenrep®

Nature: Polypeptide monoclonal antibody conjugate

Source: Synthetic conjugate inspired from dolastatin 10

Mechanism: Microtubules stabilizer {anti-mitotic}

Medical uses: Relapsed/refractory multiple myeloma

Lurbinectedin - Zepzelca®

Name: lurbinectedin - Zepzelca®

Nature: Tetra-hydroisoquinoline alkaloid

Source: Semisynthetic compound inspire from Trabectidin

Mechanism of action: DNA alkylating agent.

Medical uses: Metastatic small cell lung cancer (SCLC).

Trabectidin – Yondlis

Name: Trabectidin – Yondlis

Nature: Tetra-hydroisoquinoline alkaloid

Source: Isolated from a tunicate *Ecteinascidia turbinata.* {Now Semisynthetic}

Mechanism: DNA alkylating agent.

Medical uses: Colo-rectal cancer {Concentrated mainly in large intestine}.

Omega-3 fatty acids {PUFAs}

Name: Omega-3 fatty acids esters {DHA and EPA}

Nature: Fatty acids esters {Lipids}

Source: Cod fish liver

Mechanism: Share in biosynthesis of the useful ecosonoids - anti-oxidant

Medical uses: Anti-oxidant, anti-inflammatory and cardiovascular protective

Omega-6 fatty acids share in biosynthesis of the harmful ecosonoids

Pseudopterosine A

Name: Pseudopterosine A

Nature: Diterpene phenol

Source: Soft coral *Pseudoterogorgia elisabethae*

Mechanism: Acts by inhibition of COX I and COX II

Medical uses: Anti-inflammatory for skin care e.g. trauma

Marine Chemical Ecology

❑ Science deals with the chemical interactions between living marine organisms with each other and with the environment {It guides us as pharmacists to discover new drugs}

Puffer Fish, Pseudoalteromonas and Tetrodotoxin

❑ Tetrodotoxin was isolated from Puffer fish. The actual producer is certain symbiotic bacteria {Pseudoalteromonas gram –ve M.O}. Tetrodotoxin acts as a very strong sodium channel blocker. It is converted into a pain killer medicine Halneuron™ by WEX Pharmaceuticals Canada. It is 3000 times more active than morphine.

Cone Snail, Fish and ω-Conotoxin

❑ Snail called *Conus magus* in Philippine attacks fish by injecting mixture of cyclic peptides into fish, paralyzing them then predate on them. The major bioactive polypeptide was ω-conotoxin block voltage-gated calcium channels in neurons. The synthetic analogue of ω-conotoxin {Ziconotide - Prialt} was produced and marketed now in year 2004. It is used to treat severe chronic pain in people who cannot use or do not respond to standard pain-relieving medications.

Dolabella auricularia, Cyanobacteria and Dolastatin 10

❑ Dolastatin 10 was isolated for the first time from *Dolabella auricularia*. Evolution scientists said that nudibranchs were developed from snails losing their shells, starting produce toxic compounds as deference mechanism against enemies. Dolastatins, including dolastatin 10 recently isolated from blue green alga. Blue green algae produce these compounds as anti-deterrent against *Dolabella auricularia*. Brentuximab, a very strong and selective drug used for lymphoma was inspired from Dolastatin 10.

Cyanobacteria or Blue green algae

❑ Scientists debate about the nomenclature of Cyanobacteria to be canobacteria or blue green algae? Some scientists named them Cyanobacteria as the cell wall consisting of peptidoglycans {like bacteria} and a mixture of pigments. Other scientists named them blue green algae as they contain chlorophyll {green pigments} and Phycobillin {Blue pigments} carry out photosynthesis using chlorophyll.

Sea Hare, Blue Green Algae and Aplysiatoxin

❑ Aplysiatoxin *isolated* from the gut of *Stylocheilus longicauda*, a *sea hare*, then from the blue green algae. Blue green algae produce Aplysiatoxin as anti-predator against sea hare. Scientists confirmed the true producer by isolation of the gene cluster from the Cyanobacteria. Aplysiatoxin in small dose activates protein kinase C {PKC} leading to cell death. Aplysiatoxin is a tumor promoter in large dose, and in small dose it is anti-tumor.

Nudibranchs, Red Algae and Polyhalogenated Monoterpenes

❑ Polyhalogenated monoterpene compounds were isolated from the gut of certain nudibranchs {*Phyllidia coelestis*}. polyhalogenated monoterpene compounds were recently isolated from the red alga "*Plocamium cartilagenum*". Red algae produce these compounds ad anti-deterrant against nudibranchs. Polyalogenated monoterpene compounds showed strong cytotoxicity {5 times active} than taxol against breast cancer cell lines.

Brine Shrimp Eggs, Alteromonas and Fungi

❑ Brine shrimp eggs hatch in ocean water by 80% in the normal environmental conditions. when washed by penicillin then leaving them in ocean water, this results in 0% hatching. washing eggs with penicillin then adding the gm-ve rods micro-organisms extract named *Altermonas* with ocean water, 60% hatching was obtained. Bacteria named *Altermonas* and some fungi were grown on the microbial culture of brine shrimp eggs. bacteria is killing the fungi and eggs hatch. absence of *Altermonas* bacteria, fungi will infect Brine Shrimp eggs and they will not hatch. a compound named Pentabromo-pseudalin with very high anti-fungal activity in-vitro was isolated from *Altermonas*.

Stypopodium, snail killing Activity and Stypoldione

❑ Snails are not feeding on the brown alga *Stypopodium zonale*. The isolated compound stypoldione {Diterpenoid} was found to have ichtyotoxic activity = killing snails}. Stypoldione has sodium channel blocking activity {Neurotoxin}. It can be used as a drug lead in treatment of pain and convulsion resulting from epilepsy.

Main Problems in Marine Drug Discovery

❑ Material supply is a main problem in marine drug discovery research. marine organisms only produce low yields of bio-active compound.

❑ To obtain 1 gm of trabectedin, a drug for sarcomas, colorectal and ovarian cancer, approximately 1 ton of the tunicate should be extracted. A reliable source of material for drugs development is essential. Alternative solutions to overcome the problems are urgently needed. Alternative solutions to this problem.

Alternative Sources of the Rare Bioactive Marine Natural Compounds

1 - Aquaculture for sustainable supplies of Marine-Derived Drugs Material

Trabectedin {Yondelis™] was isolated from *Esteinascidia turbinata*. *Esteinascidia turbinata* is a tunicate normally lives in shallow waters Caribbean coastal. Trabectedin (Yondelis™) was reported to have a unique anti-cancer mechanism of action. It binds to the DNA and induces transcription inhibition. Trabectedin (Yondelis ™) was approved as anticancer drug by FDA in 2014. Working with structurally complex compounds where total or even semi synthesis is very difficult to be provided, aquaculture can be a solution. Aquaculture of *Esteinascidia turbinata*, is a success example of how aquaculture can be used to supply material for promising marine drugs candidate. Marine natural products isolated from tunicates, sponges or other marine invertebrate usually have a very complex structural complexity which are not feasible to be produced by total synthesis. Total synthesis of Trabectedin (Yondelis™) was completed but it is costly and needs 18 steps. However, it was not sufficient to be the only source for clinical development needs. PharmaMar, a pharmaceutical company developed Trabectedin decided to use natural sourcing as main material resource for pre-clinical and clinical needs. Finally, PharmaMar decided to switch the source of Trabectedin (Yondelis™) production from natural resources to semi-synthetic source as they isolated safracin B from a microorganism lives in symbiosis with the marine tunicate.

2 - Chemical Synthesis of Marine-Derived Drugs (Ziconotide - Prialt™)

Developing chemical synthesis is the main approach that currently used to produce marine-derived drugs candidates. One of case example of the success used of this approach is the chemical synthesis of ziconotide. The lead compound of ziconotide was ω-conotoxin which was isolated from cone snail.

ω-conotoxin is a polypeptide consists of 27 amino acids. Chemical synthesis approach is feasible solution to be used, especially when working with less complex structure of compounds e.g. Cytarabine^{TR} and Ziconotide^{TR}.

Eribulin is another example of totally synthetic marine drugs as:

a. Aquaculture of *Halichondria* is an insufficient source for the Halichondrin B

b. Microbe(s) responsible for production of Halichondrin B couldn't be cultured

c. Gene cluster for biosynthesis of Halichondrin couldn't be isolated from the true producers till now. Halichondrin B produced by the association between the different symbiont microorganisms.

Trabectedin is semi-synthesized from Safracin B, a microbial metabolite from *Pseudomonas fluorescens*

3- Biotechnology in Marine Drug Discovery

❑ Compounds isolated from marine invertebrate were originating from endo-symbiotic microorganism.

❑ Numerous data showed that the bioactive compounds are produced either by the symbiont or by the association between the host and symbiont or by the association between the different symbiont microorganisms. Halichondrin B, a highly bioactive anti-mitotic macrolide {Tubulin assembly inhibitor} produced by associated microorganisms live in symbiosis with marine sponge *Halichondria okadai*.

❑ A new strategy of culture-independent method (Marine biotechnology) to explore the untapped reservoir of marine microbial chemical diversity has been developed. Metagenomics marine biotechnology is basically a modern genomics approach to isolate the DNA of microbial communities directly from their natural environments; therefore difficulties associated with culturing the microbes can be avoided.

❑ Since the genetic information that encoding the secondary metabolites produced by microorganism is typically clustered on the microorganisms' chromosomes, hence it is possible to predict the complete gene clusters of secondary metabolites biosynthesis on those organisms, which might provide a renewable source of the secondary metabolites. Once a gene has been identified, the genetic information can be used for compound production. Biotechnology can be simply defined as making other organisms work for us.

❑ Marine biotechnology is a rising area in Marine drug discovery.

❑ Marine Biotechnology is considered as the future work of marine natural products {marine drugs}. Marine Biotechnology includes Isolation of the gene cluster of the bioactive compound by isolation of the DNA and/or mRNA. Production of the bioactive compounds by using recombinant DNA technique. Production of new compounds with unique novel bioactivities by using mixed recombinant DNA.

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Biotechnology in Marine Drug Discovery

❑ Genetic information encoding the secondary metabolites produced by organism is typically clustered on the organisms chromosomes. It is possible to predict the complete gene clusters of secondary metabolites biosynthesis on those organisms, which might provide a renewable source of the secondary metabolites. Genetic information can be used for compound production, once a gene has been identified.

❑ Marine biotechnology deals with separation of gene clusters of biosynthetic pathways of bioactive compounds to be transferred to E. coli or yeast for in-vitro production of these compounds by genetic engineering.

❑ Marine biotechnology is a rising area in marine drug discovery.

Transgenic micro-organisms: A bacterium or fungus into which genetic information {Recombinant DNA} has been introduced from the outside e.g. *E. coli*.

Transgenic plants: A plant {Nicotiana – Soybean} into which Recombinant DNA has been introduced from the outside to create plants with new characteristics.

Molecular farming: Technology uses plants to produce large quantities of pharmaceutical substances like antibiotics, vaccines and antibodies

Overcoming Bacterial Resistance: Technology uses transgenic plants to produce large quantities of phage lysins, to use these lysins beside antibiotics. By this combination phage lysins will facilitate the entrance of antibiotic to resistant bacterial cell causing bacterial cell death

Phage lysins: Bacterial cell wall hydrolytic enzymes {peptidoglycan lyases} used to rupture bacteria. They are synthesized and sequestered with assembling phage particles.

Diagnostic fluorescent compounds from marine jellyfish: Coelenterazine from jellyfish in medical diagnostics e.g. determining cardiac output, liver blood flow and for ophthalmic angiography.

Cell, DNA, Gene and Genome

DNA: Deoxyribonucleic acid is the molecule that carries the genetic information in all cellular forms of life and some viruses.

Gene: Specific sequence of nucleotides at a given position on a given chromosome that codes for a specific protein (or mRNA molecule).

Genome: Includes all of its genes. In humans, a copy of the entire genome more than 3 billion DNA base pairs is contained in all cells that have a nucleus.

Cell, DNA, Gene and Protein

Proteins: Large molecules composed of one or more chains of amino acids in a specific order determined by the base sequence of nucleotides in the DNA.

Genetic code: The sequence of nucleotides in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that determines the amino acid sequence of proteins.

Codons: Every triplet of nucleotides in a nucleic acid sequence specifies a single amino acid.

Universal Genetic code: Same code is used by bacteria, plants and humans. gene encoding a plant protein can be inserted into a bacterium.

DNA is not directly involved in protein synthesis

Protein synthesis is a two-step process that involves two main events called transcription and translation. mRNA is transcribed into a sequence of amino acids. When reading the mRNA, it is "read" in a series of three adjacent nucleotides.

Restriction Enzymes {Restriction endonuclease}: Protein produced by bacteria that cleaves DNA at specific sites along the molecule. In the bacterial cell, restriction enzymes cleave foreign DNA, thus eliminating infecting organisms. Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason they are indispensible tools of recombinant DNA technology (genetic engineering).

Genetic engineering, {Recombinant DNA technology}: Group of techniques used to cut up and join together genetic material, especially DNA from different biological species, and to introduce the resulting hybrid DNA into an organism in order to produce a pharmaceutically important compound on large scale by the second organism.

Introduction of a gene { Recombinant DNA} into plant Agrobacterium tumifaciens: A gram -ve soil bacteria that works as a natural genetic engineer. It can invade plants through wounds in the stem or root. These bacteria have an extra piece of DNA called the Ti plasmid. Once inside the plant, a portion of the Ti plasmid will leave the bacteria, enter the plant cell and insert itself into the plant's chromosomes. Genes in *Agrobacterium tumifaciens* encode enzymes for auxin and cytokinin enable *Agrobacterium* cause crown gall. Genetic engineers have removed the genes from the Ti plasmid that alter metabolism and cause disease and in their place incorporated desired genes. These bacteria with their altered plasmids can be used to infect and transform plant cells growing in tissue culture and produce the compound of interest.

Gene guns {Biolistic particle delivery system}: used to deliver exogenous DNA (transgenes) to cells. The gold nano particles with gene or recombinant DNA loaded into gene gun, fires them at a suspension of cultured cells

Electroporation: Use of high-voltage electric shocks to introduce genes into cells. Allows the generation of genetically modified cells.

Very short, high-voltage {Thousends of volts} electric pulses applied. Small organic metabolites to large macromolecules can be introduced.

Gene Hunting

- Gene hunting is the process of finding genes that contribute to biosynthesis of certain bioactive compound.

Finding a particular gene by creating a complementary DNA (cDNA)

- Complementary DNA (cDNA) is the DNA produced on an RNA template by the action of reverse transcriptase (RNA-dependent DNA-polymerase).
- The sequence of the cDNA becomes complementary to the RNA sequence .

Steps of Gene Hunting:

- Isolate messenger RNA (mRNA) from cells that contain the gene.
- Reverse transcriptase is mixed with this isolated mRNA.
- Reverse transcriptase uses mRNA as a template make single strand of DNA {cDNA}.

Polymerase chain reaction (PCR)

- Double strand DNA can be obtained by DNA Polymerase from cDNA
- *in-vitro* amplification of specific sequences of DNA Using *Taq* DNA polymerase.

Basic steps of PCR

- De-naturation by heating of a template DNA molecule to be copied.
- Annealing of pairs of nucleosides of specific sequences.
- Extension by DNA polymerase from primers to copy the template DNA molecule.

Steps to produce a pharmaceutically active compound by biotechnology

- Isolation and structural elucidation of the bioactive compound from marine organism.
- Studying the biosynthesis of marine natural products like polypeptides compounds.
- Isolation of gene cluster of the bioactive compound isolation DNA and/or mRNA.
- Purification of gene cluster by Gel electrophoresis,
- PCR and sequencing of the cDNA.
- Construction of the recombinant DNA.
- Production of compound by cell culturing or plant tissue culture.
- Production of new compounds with unique novel bioactivities by using mixed recombinant DNA.